British Journal of Applied Science & Technology
7(1): 62-83, 2015, Article no.BJAST.2015.126
ISSN: 2231-0843

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Study of Thermal Behaviour of Milk Protein Products Using a Chemometric Approach

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Authors' contributions

This work was carried out in collaboration between all authors. Author RS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors MRA, MALO, LFCO, ITP, PHFS managed the analyses of the study, literature searches and in the discussion of the results. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2015/15372
Editor(s):
(1) Soichiro Nakamura, Department of Bioscience and Biotechnology, Shinshu University, Japan.
(2) Harshadrai M. Rawel, Institute of Nutritional Science, University of Potsdam, Germany.
Reviewers:
(1) Anonymous, Malaysia.
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Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=770&id=5&aid=7832

Original Research Article

Received 22nd November 2014
Accepted 2nd January 2015
Published 21st January 2015

ABSTRACT

This study deals with the evaluation of the behavior of viscosity evolution of the Milk Protein Products (MPPs) solutions in different concentrations used in different conditions of thermal processing by means of Rapid Visco Analyzer (RVA). Skimmed milk powder (SMP), whey protein

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concentrate (WPC), milk protein concentrate (MPC), whey powder (WP) and demineralized whey powder (DWP) were analyzed; the evaluated solid-liquid ratio solutions were 0.1 $g \cdot g^{-1}$ to 0.45 $g \cdot g^{-1}$ of dry solids in water (DSW) for the MPC, 0.1 $g \cdot g^{-1}$ to 0.4 $g \cdot g^{-1}$ of DSW for the WPC and 0.5 $g \cdot g^{-1}$ to 0.8 $g \cdot g^{-1}$ of DSW for SMP, WP and DWP. Brazilian industrial heat treatment set ups were used, with temperatures ranging from 65°C to 95°C and retention time from 5 to 30 minutes. The results were interpreted according to the viscosities during heating and cooling, being possible to optimize the MPPs different parameters conditions with the interpretations of the viscographic profiles based on the thermal behavior of proteins, especially for the SMP, MPC and WPC. The data showed similarity in viscographic characteristics of the solutions when evaluated at 65°C for 30 minutes, at 85°C for 15 minutes and 95°C for 5 minutes, for the MPC and WPC at 95°C for 5 minutes, all of them for different concentrations of proteins in water.

Keywords: Milk protein; functionality; viscosity.

ABBREVIATIONS

MPPs = milk protein products; RVA = rapid visco analyzer; SMP = skimmed milk powder; WPC = whey protein concentrate; MPC = milk protein concentrate; WP = whey powder; DWP = demineralized whey powder; MPC1 = solutions of milk protein concentrate with prior hydration; MPC2 = solutions of milk protein concentrate without prior hydration; DSW= dry solids in water; TCW = thermocline for windows; PV = peak viscosity; RV = retention viscosity; FV = final viscosity; ∫/t = integration of viscographic profile divided by the total time of analysis; PCA = principal component analysis; PC1 = First principal component; PC2 = second principal component; β-LG = β-lactoglobulin; α-LA = α-lactalbumin.

1. INTRODUCTION

Dairy protein products can be defined as concentrated or dried milk products that whenever added to dairy products or other processed food as ingredient contribute to the improvement of the nutritional composition and also to functionality during and after processing [1].

During the last years the production of dairy protein powder is increasing mainly for baby food production, for special diets, animal feeding and for the food industry applications. Membrane separation processes, vacuum evaporation and spray drying are mandatory steps in the production of dairy proteins powders. The dairy industry has developed new technological processes for extracting and purifying proteins such as milk protein concentrate (MPC), milk protein isolate (MPI), whey protein concentrate (WPC), whey protein isolate (WPI), micellar casein concentrates (MCC) and isolates (MCI), whey concentrates and selectively demineralized whey concentrates [2].

The process of converting liquid milk or whey into powder alters the nature and behavior of milk components. Factors which affect the ability of water absorption by milk proteins include: Composition, protein structure and conformation, surface load and polarity, presence of carbohydrates, lipids and salts, pH, ionic strength, temperature, degree of denaturation and aggregation, and formation of disulfide bridges [3,4].

The understanding of the involved interactions and evaluation of the effect of milk proteins as industrial ingredient still needs more studying so as to develop a better suitability of the use of these proteins within each desired technological application in the various processing lines. The understanding of the interaction of milk proteins provides the food industry the formula to obtain a relationship which delivers a better cost/benefit ratio by using each type of protein within the desired characteristics in the final product, thus bringing to market products that use as technological ingredients, proteins with high biological value [5].

The Rapid Visco™ Analyser (RVA™) is a rotational viscometer capable of continuously measure the viscosity of a sample under controlled temperature [6].

This study has as goal to investigate the influence of different thermal processing conditions on the evolution of the viscosity of dehydrated dairy protein after adequate

rehydration, using the Rapid Viscosity Analyzer (RVA) as simulator.

2. MATERIALS AND METHODS

Five types of dehydrated MPPs for evaluation were selected, being the selection criteria the industrial use in Brazil, as well the national industrial production, current economic importance, the extent of use in different areas and prospecting for future use [7].

Therefore, a sample of industrial production of each MPP was previously selected in the national market or overseas, taking into consideration the quality of products and manufacturing date. The products were acquired and kindly provided by Gemacom Tech Company. The products had their chemical and microbiological characteristics previously informed by their manufacturers through analysis of reports of purchased lots.

Analyses were conducted to determine the results showed by reports at Gemacom Tech Laboratory of Product Analyzes. Table 1 shows every analysis carried out.

Three processing levels (low, medium and high) were used to simulate different thermal processing for each MPP sample, which were selected based on their usefulness in the processing of cheese, fermented milk, Petit Suisse, pasteurized cream, and flavored milk drinks: 65°C for 30 minutes (low), 85°C for 15 minutes (medium) and 95°C for 5 minutes (high). It is important to notice that the three heat treatment processes above used in this investigation are the most usual parameters used by Brazilian industries. As recommended by [8], all samples were previously hydrated before being subjected to the RVA. Genuine replicates of the solutions were prepared in duplicates using distilled water as solvent and constant stirring for 30 min at 25°C. All these solutions have been prepared with 100 g water and sufficient amount of MPP to obtain the desired concentration of dry basis (dry basis g/water g). The dehydrated products were kept in airtight containers due to the hygroscopic characteristics of the samples, avoiding, thus, absorption of humidity. Before the preparation of each solution, moisture analyzes were performed to obtain the exact calculation of the amount of MPP to be used for its concentration of dry basis. The concentrations of evaluated solutions of each product are shown in Table 2.

Due to the solubility characteristics of the MPC, thermal processing simulations were also performed without prior hydration, adding this MPP directly to water already in the tank of the equipment. Abbreviations MPC1 and MPC2 refer, respectively, to solutions of MPC with and without prior hydration. In the simulations, 25 g of each solution were weighed beforehand in the heating tank of RVA. In the case of MPC, without prior hydration, calculations were made for the solution prepared directly in the bowl also presented final mass of 25 g. To ensure full standardization of solutions, RVA rotation was set to 104.7 $rad \cdot s^{-1}$ during the first 60 seconds at constant temperature of 30°C. The SMP, WP, DWP and WPC solutions had the pH adjusted to 6.5 using 1 $mol \cdot L^{-1}$ of sodium hydroxide solution. After dissolution, the pH of MPC solutions ranged from 6.7 to 6.9, and to latter there was no conduction to pH adjustment. The RVA equipment used in this work was the Model Series 4 (RVA-4) from NEWPORT SCIENTIFIC. The software used for data collection was the Thermocline for Windows, TCW. The configurations of the thermal processing simulations are shown in Table 3.

The gradients of heating and cooling were also standardized between the levels, being 6.5°C per minute to 13.0°C per minute respectively. Integration calculations of viscographic profiles were performed in MICROCALTM ORIGIN® version 6.0 software. All integration calculations were made with the viscographic profiles as from 67 seconds, discarding, this way, the stirring time at a speed of 104.7 $rad \cdot s^{-1}$ as described in step preparation of samples. It was determined in the experiment that the highest viscosity obtained during thermal processing before the start of cooling would be represented as peak viscosity (PV), the viscosity obtained at the end of the retention time of thermal processing would be represented as retention viscosity (RV), the last viscosity reading obtained at the end of the analysis would be represented as final viscosity (FV) and the value of the integration of viscographic profile divided by the total time of analysis would be represented as area per unit time ($\int/t$). To study the optimization of the concentration, temperature and time in simulated conditions of thermal processing in RVA, the WPC was selected among the available MPPs for evaluations since it is a high protein product and widely utilized in food, as well as an ingredient which assigns functionality properties.

A 3^3 Box-Behnken factorial design was performed to evaluate the effect of thermal processing on the characteristics of MPPs by monitoring the final viscosity (FV) through the solutions obtained after complete cycle of heating and cooling. The selected factors are shown in Table 4.

Table 5 presents the matrix of planning, with the levels and factors combinations, where the experiments 13, 14 and 15 are replicates of the center point. The procedures were performed randomly by raffle.

Table 1. Methods of analysis for each parameter of MPP

Analysis	Milk derivate		Whey derivate		
	Skimmed milk powder	Milk protein concentrate	Whey powder	Demineralized whey powder	Whey protein concentrate
Total fat	IN n° 68 de 12/12/2006	AOAC 989.05	FAO, 1976	FAO, 1976	AOAC 989.05
Moisture	FIL 26A: 1993	AOAC 925.45	FIL 26A: 1993	FIL 26A: 1993	AOAC 927.05 17th
Total protein	IN n° 68 de 12/12/2006	AOAC 991.20	FIL 20B: 1993	FIL 20B: 1993	AOAC 991.20
Protein in dry basis	Calculated	Calculated	Calculated	Calculated	Calculated
Lactose	Calculated	Calculated	Calculated	Calculated	Calculated
Ash	IN n° 68 de 12/12/2006	AOAC 900.02	AOAC 15th, 1990	AOAC 15th, 1990	AOAC 900.02
Acidity	FIL 81:1981	FIL 81:1981	FIL 81: 1981	FIL 81: 1981	n.a.[1]
Chloride	n.a.[1]	n.a.[1]	n.a.[1]	LANARA	n.a.[1]
pH	n.a.[1]	AOAC 981.12	Adolfo Lutz	Adolfo Lutz	AOAC 981.12
Specific mass	n.a.[1]	C314 VK Density Tapper	Niro Atomizer	Niro Atomizer	C314 VK Density Tapper
Total count of mesophilic aerobic	FIL 100B:1991	AOAC 989.10	FIL 100B:1991	FIL 100B:1991	AOAC 989.10
Coliforms at 30°C	APHA 1992	AOAC 989.10	APHA 1992	APHA 1992	AOAC 989.10
Escherichia coli	APHA 1992	AOAC 989.10	APHA 1992	APHA 1992	AOAC 989.10
Fungi and yeasts	FIL 94B: 1990	AOAC 989.10	FIL 94B: 1990	FIL 94B: 1990	AOAC 989.10
Coagulase positive staphylococci	FIL 138:1986	AOAC 2003.08	FIL 138:1986	FIL 138:1986	AOAC 2003.08
Salmonella sp	FIL 93B:1995	AOAC 967.25	FIL 93B:1995	FIL 93B:1995	AOAC 967.25

[1] *not analysed*

Table 2. Solution concentrations of the MPPs evaluated in RVA

Product	Concentration $g \cdot g^{-1}$ (dry basis)	
	With prior hydration	Without prior hydration
Skimmed milk powder (SMP)	0.5 / 0.6 / 0.7 / 0.8	X
Milk protein concentrate (MPC)	0.1 / 0.12 / 0.14 / 0.16	0.3 / 0.35 / 0.4 / 0.45
Whey powder (WP)	0.5 / 0.6 / 0.7 / 0.8	X
Demineralized whey powder 40% (DWP)	0.5 / 0.6 / 0.7 / 0.8	X
Whey protein concentrate (WPC)	0.1 / 0.2 / 0.3 / 0.4	X

Table 3. Settings of thermal processing employed in RVA

Time	Set up	65°C for 30 minutes	85°C for 15 minutes	95°C for 5 minutes
0 min 00 s	Temperature (°C)	30	30	30
0 min 00 s	Rotation ($rad.s^{-1}$)	104.7	104.7	104.7
1 min 00 s	Rotation ($rad.s^{-1}$)	15.7	15.7	15.7
1 min 00 s	Temperature (°C)	30	30	30
6 min 24 s	Temperature (°C)	65	85	95
36 min 24 s	Temperature (°C)	65	85	95
39 min 06 s	Temperature (°C)	30	30	30
44 min 06 s	Temperature (°C)	30	30	30
44 min 07 s	End	X	X	X

For all three treatment levels, the gap between viscosity readings was 4 seconds

Table 4. Factors and levels used in the 3^3 Box-behnken factorial design

Factors	Factors levels		
	-1	0	+1
Time (minutes)	5	15	30
Temperature (°C)	65	85	95
Concentration ($g \cdot g^{-1}$)	0.2	0.3	0.4

Table 5. Coded matrix for the WPC 3^3 box-behnken design

Experimental set randomized	Time (level)	Time (minutes)	Temperature (level)	Temperature (°C)	Concentration (level)	Concentration ($g \cdot g^{-1}$)
10	0	15	1	95	-1	0.2
6	1	30	0	85	-1	0.2
3	-1	5	1	30	0	0.3
12	0	15	1	95	1	0.4
4	1	30	1	95	0	0.3
2	1	30	-1	65	0	0.3
14	0	15	0	85	0	0.3
5	-1	5	0	85	-1	0.2
8	1	30	0	85	1	0.4
13	0	15	0	85	0	0.3
15	0	15	0	85	0	0.3
1	-1	5	-1	65	0	0.3
7	-1	5	0	85	1	0.4
9	0	15	-1	65	-1	0.2
11	0	15	-1	65	1	0.4

2.1 Data Analysis

The obtained results were analyzed using the coefficient and regression correlation in order to conclude on the definition of the main factors to be used in interpreting the viscographic profile of MPPs in simulated conditions in RVA. Analyses of variance were also conducted to test differences in the studied variables with MPPs in relation to the concentration and thermal processing. It was applied the system for statistical analyses SAEG 9.1. For the regression analysis, models that had a significance level less than or equal to 5% were accepted as model and as linear and quadratic coefficients, when it was the case. For the analysis of correlation coefficients were accepted with a significance level equals or less than 5%. The response surfaces and calculations of the effects were obtained by employing the computer program STATISTIC 6.0 (Statsoft, Inc.) and ANOVA, Microsoft Excel 2007 (Microsoft Co.). For the exploratory analysis by type of thermal processing among the MPPs, the results of the RVA were organized into matrices with treatments (MPP and concentration) in the lines and the viscosity readings averages from all viscographic profile in columns and then submitted to the Principal Component Analysis (PCA) from the covariance matrix, using PLS Toolbox v.2.9.2.9 (Eigen Vector Research Inc., Wenatchee, WA), operating in MATLAB (R2007B, vol. 7.5.0.342, Inc . the Mathworks, Natick, MA) environment.

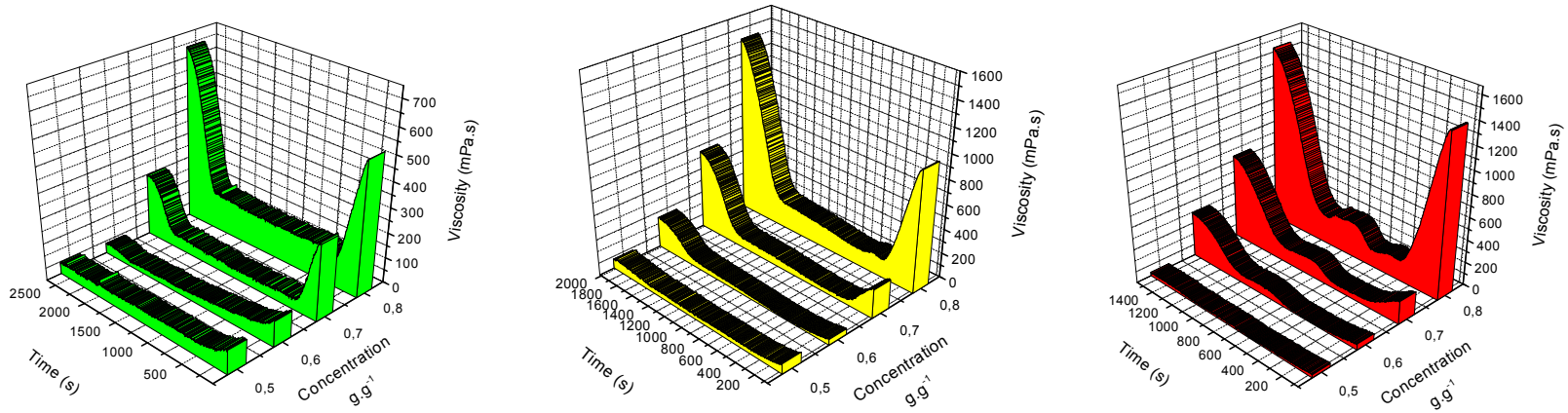
3. RESULTS AND DISCUSSION

The samples collected and used in this study presented their compositional and microbio-logical characteristics consistent with data from literature [1]. The Table 6 presents the MPP's chemical and microbiological composition.

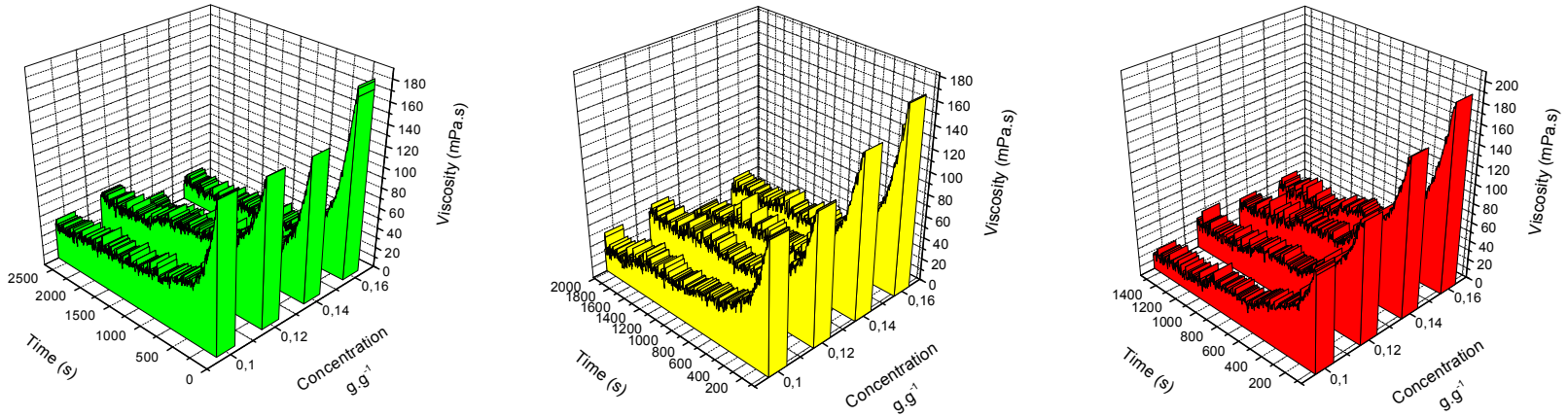
The viscographic profiles of simulated thermal processing in RVA for the MPP´s are shown in Fig. 1 respectively to low, medium and high heat treatment.

The analysis of variance for PV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.003$) in SMP. These results demonstrate the influence of these two variables in the maximum viscosity obtained during the process provided by the SMP. The maximum value of PV obtained for SMP was 318 mPa·s on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and less than 27 mPa·s with 0.6 $g \cdot g^{-1}$subjected to 65°C for 30 minutes. The maximum value of RV for SMP was 308 mPa·s on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum 1mPa·s with 0.6 $g \cdot g^{-1}$ subjected to 65°C during 30 minutes.

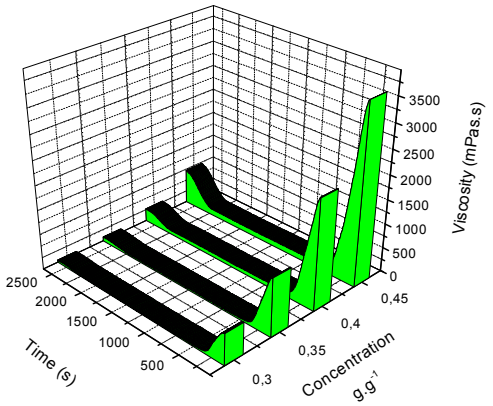
Skimmedmilk poder (SMP)

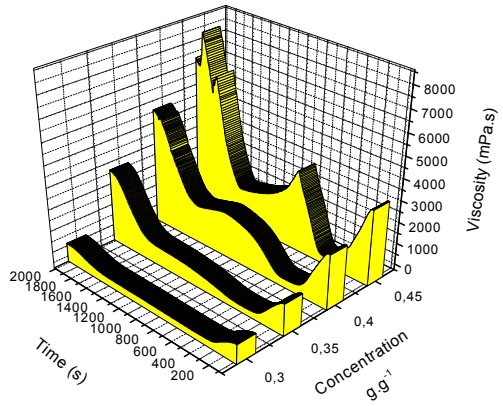


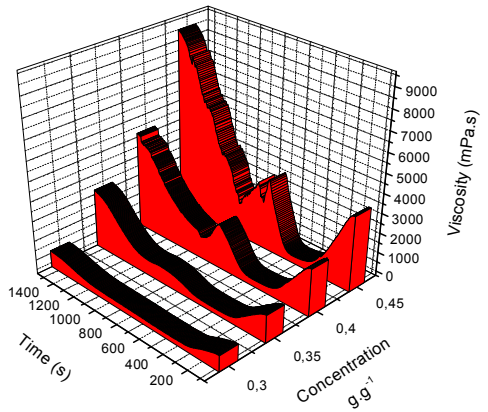
Milk protein concentrate (MPC_1)



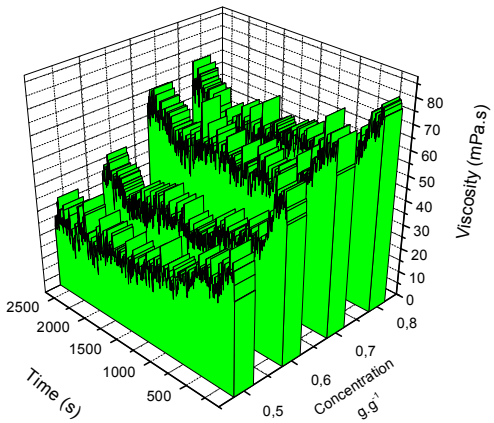
Milk protein concentrate (MPC_2)

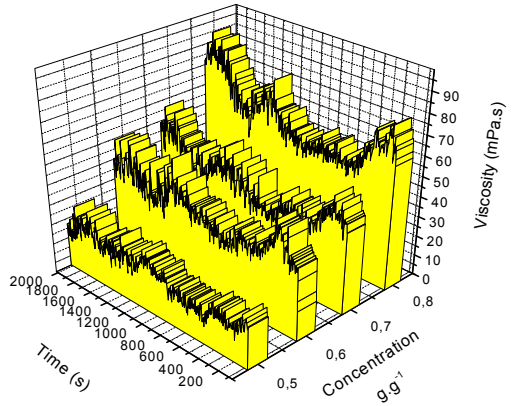


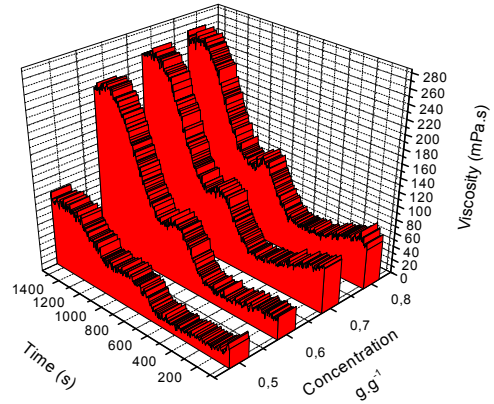




Whey powder (WP)







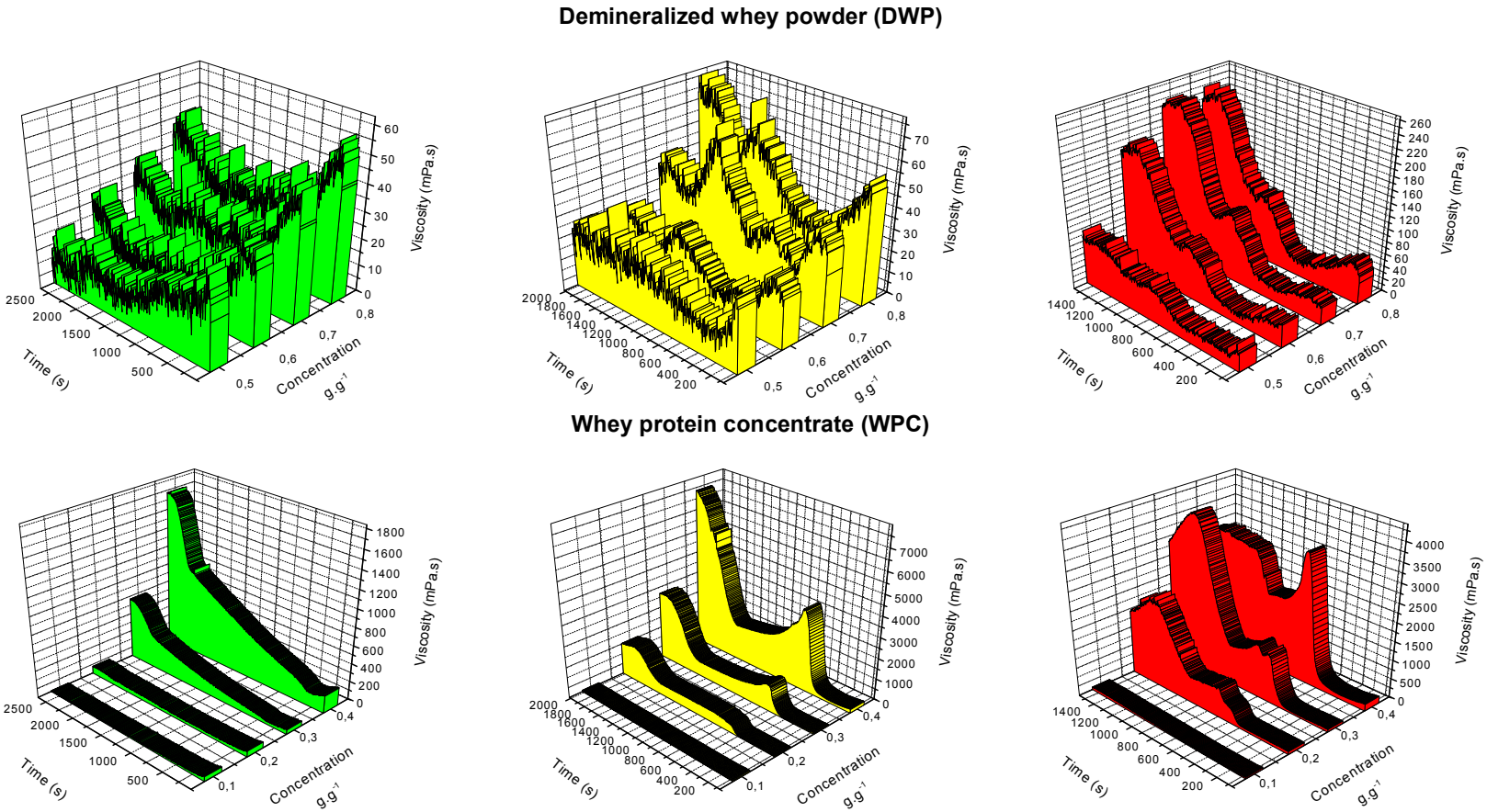


Fig. 1. Viscografic profiles of simulate thermal processing in RVA for the MPPs respectively for low (γ), medium (γ) and high heat treatment (γ)

Table 6. Microbiological and chemical composition of MPP

Analysis	Milk derivate		Whey derivate		
	Skimmed milk powder	Milk protein concentrate	Whey powder	Demineralized whey powder	Whey protein concentrate
Total fat ($g \cdot kg^{-1}$)	15.9	9.7	15	5	61.5
Moisture ($g \cdot kg^{-1}$)	32.5	42.7	18	17	33.9
Total protein ($g \cdot kg^{-1}$)	351.4	714.3	123.6	122.8	785.3
Protein in dry basis ($g \cdot kg^{-1}$)	363.2	746.2	125.9	124.9	812.8
Lactose ($g \cdot kg^{-1}$)	511	161.3	768.9	795.9	95.7
Ash ($g \cdot kg^{-1}$)	89.2	72.0	74.5	59.3	23.6
Acidity (g ácido lático$\cdot L^{-1}$)	1.4	1.5	1.7	1.4	n.a.[1]
Chloride ($g \cdot kg^{-1}$)	n.a.[1]	n.a.[1]	n.a.[1]	10.8	n.a.[1]
pH	n.a.[1]	6.95	6.13	6.10	6.33
Specific mass($g \cdot mL^{-1}$)	n.a.[1]	0.52	0.74	0.63	0.43
Total count of mesophilic aerobic	$2.6 \cdot 10^{3}$ UFC$\cdot g^{-1}$	$9.6 \cdot 10^{3}$ UFC$\cdot g^{-1}$	$4.2 \cdot 10^{3}$ UFC$\cdot g^{-1}$	$3.9 \cdot 10^{3}$ UFC$\cdot g^{-1}$	$7 \cdot 10^{2}$ UFC$\cdot g^{-1}$
Coliforms at 30°C	<10 NMP$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$	<10NMP$\cdot g^{-1}$	<10 NMP$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$
Escherichia coli	<1 NMP$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$	<3 NMP$\cdot g^{-1}$	<1 NMP$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$
Fungi and yeasts	<10 UFC$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$	40 UFC$\cdot g^{-1}$	<1 UFC$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$
Coagulase positive staphylococci	<10 UFC$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$	<1 UFC$\cdot g^{-1}$	<1 UFC$\cdot g^{-1}$	<10 UFC$\cdot g^{-1}$
Salmonella sp	absent in25g	absent in 25g	absent in 25g	absent in 25g	absent in 25g

[1] *not analyzed*

For the same product the maximum value of FV was 1645 mPa·s on the condition of 0.8 $g \cdot g^{-1}$ at 85°C for 15 minutes and less than 19 mPa·s with 0.5 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The magnitude of the effects of concentration and thermal processing on PV, RV and FV clearly manifested ($P<0.05$), being the concentration of 0.8 $g \cdot g^{-1}$ and binomial 95°C for 5 minutes and 85°C for 15 minutes the most likely to show higher values of viscosity.

The analysis of variance for ∫/t showed statistically significant effects for concentration ($P <0.001$) and thermal processing ($P < 0.001$). These results demonstrate the influence of these variables in the full viscographic profile of SMP. The maximum value of ∫/t obtained in the study was 563 on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and 0.6 with at least 16 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The evaluation of the magnitude of the effects of concentration and thermal processing on PV, RV and FV can be made to ∫/t, demonstrating be statistically significant ($P < 0.05$), with a concentration of 0.8 $g \cdot g^{-1}$ and 95°C binomials for 5 minutes and 85°C for 15 minutes more likely to display higher values of viscosity (see Table 7).

In the case of MPC1 analysis of variance for PV revealed statistically significant effect for thermal processing ($P < 0.001$). The maximum value of PV obtained in the study was 84 mPa·s on the condition of 0.12 $g \cdot g^{-1}$ at 65°C for 30 minutes and the minimum 39 mPa·s with 0.1 $g \cdot g^{-1}$ subjected to 95°C for 5 minutes. The maximum concentration to be prepared from the solution of MPC1 with the same procedures performed for other products was 0.16 $g \cdot g^{-1}$. This occurred due to the large increase in viscosity at 25°C, making it impossible to maintain agitation of the solution within 30 minutes, which interfered in its uniformity. The main factor affecting the solubility of MPC1 seems to be related to the throughput of water in the powder particle more than the thermal processes during manufacture [9]. Different methods for producing MPC1 with high solubility in cold water have been proposed, involving the addition of monovalent salts in the retentate prior to drying [10] or partial replacement (approximately 30%) of the calcium content of the retentate sodium ions by ultrafiltration [11]. MPC1 analysis of variance for FV showed a statistically significant effect for concentration ($P < 0.001$). The maximum value for MPC1 of FV was 50 mPa·s on the condition of 0.12 $g \cdot g^{-1}$ at 65°C for 30 minutes and less than 10 mPa·s with 0.14 $g \cdot g^{-1}$ subjected to 85°C for 15 minutes. The viscographic profiles obtained for MPC1 in this concentration range reveal an inherent characteristic of the product which is the high viscosity before processing and significant decrease during the heat treatment, without any increase of viscosity even after cooling. These results are in agreement with the proposal made by [9], explaining the low solubility of the MPC1 at low temperatures. The magnitude of the

effects of concentration and thermal processing on the FV and PV, respectively, were statistically significant (P <0.05). However, it is possible to correlate any technological factor variables which exhibited the highest values of viscosity. The maximum value of ∫/t 59 was obtained in the study of 0.12 $g \cdot g^{-1}$ at 65°C for 30 minutes and the minimum 0.14 to 29 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The correlation data from ∫/t with viscosities PV, RV and FV for the MPC1 are shown in Table 7 and indicate the poor correlation among them. The low concentration of protein is the main reason why the results obtained do not have good correlation, because the viscosity increase in this range is small.

With regard to MPC2, the analysis of variance for PV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum value of PV for the MPC2 was 3568 mPa·s on the condition of 0.45 $g \cdot g^{-1}$ at 85°C for 30 minutes and the minimum was 20 mPa·s with 0.3 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The analysis of variance for RV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for MPC2. These results demonstrate the influence of these variables on the viscosity obtained after the retention time, provided by MPC2. The maximum value of RV obtained in the study was 2180 mPa·s on the condition of 0.45 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 20 mPa·s with 0.3 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. For MPC2 analysis of variance revealed statistically significant effects for FV for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum FV value obtained in the study was 9050 mPa·s on the condition of 0.45 $g \cdot g^{-1}$ at 95°C for at least 5 minutes and the minimum was 44 mPa·s with 0.3 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The magnitude of the effects of concentration and thermal processing on PV, RV and FV were statistically significant ($P < 0.05$) for MPC2, being the concentration of 0.45 $g \cdot g^{-1}$ and the binomial 95°C per 5 minutes and 85°C per 15 minutes the most likely to show higher values of viscosity. The analysis of variance for ∫/t showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). These results demonstrate the influence of these variables on the complete MPC2 viscographic profile. The maximum value of ∫/t obtained in the study was 3113 of 0.45 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 58 with 0.3 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The evaluation on the magnitude of the effects of concentration and thermal processing on PV and RV FV can be verified by ∫/t, demonstrating to be statistically significant ($P < 0.05$), and the concentration of 0.45 $g \cdot g^{-1}$ and the binomial 95°C for 5 minutes and 85°C for 15 minutes are the most likely to exhibit higher values of viscosity. This similarity among the results obtained for ∫/t with viscosities PV, RV and FV is deduced when analyzing the correlation coefficients shown in Table 7.

For WP, the analysis of variance for PV revealed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum value of PV obtained for WP was 126 mPa·s on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was of 30 mPa·s with 0.5 $g \cdot g^{-1}$ subjected to 85°C for 15 minutes. The analysis of variance for RV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). These results demonstrate the influence of these variables on the viscosity obtained after the retention time, provided by WP. The maximum value of RV was 117 mPa·s on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 10 mPa·s with 0.5 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The analysis of variance for FV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for the WP. The maximum value of FV was 256 mPa·s on the condition of 0.7 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 7mPa·s with 0.5 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The analysis of variance for ∫/t showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for WP. The maximum value of ∫/t obtained was 116 on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 20 with 0.5 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The evaluation on the magnitude of the effects of concentration and thermal processing on PV, RV and FV can be verified by ∫/t, demonstrating to be statistically significant ($P < 0.05$), being the concentrations of 0.8 $g \cdot g^{-1}$, 0.7 $g \cdot g^{-1}$ and 0.6 $g \cdot g^{-1}$, and the binomial 95°C for 5 minutes the ones with the largest propensity to exhibit higher values of viscosity. This similarity among the results obtained for ∫/t with viscosities PV, RV and FV is deduced when analyzing the correlation coefficients presented in Table 7.

Regarding the DWP, the analysis of variance for PV revealed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum value of PV obtained in the study was 114 mPa·s on the

condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 27 mPa·s with 0.5 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes.

The analysis of variance for RV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for DWP. The maximum value of RV obtained in the study was 110 mPa·s on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 10 mPa·s with 0.6 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The analysis of variance for FV showed statistically significant effects for concentration ($P < 0.002$) and thermal processing ($P < 0.001$) for the DWP. The maximum FV value obtained in the study was 255 mPa·s on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 9mPa·s with 0.5 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The magnitude of the effects of concentration and thermal processing on PV, RV and FV were statistically significant ($P < 0.05$) for the DWP, being the concentrations of 0.8 $g \cdot g^{-1}$ and 0.7 $g \cdot g^{-1}$, and the binomial 95°C for 5 minutes the most likely to show higher values of viscosity. The analysis of variance for ∫/t showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum value of ∫/t obtained in the study was 99 on the condition of 0.8 $g \cdot g^{-1}$ at 95°C for 5 minutes and the minimum was 15 with 0.5 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The evaluation on the magnitude of the effects of concentration and thermal processing on PV, RV and FV can be verified by ∫/t, demonstrating to be statistically significant ($P < 0.05$), being the concentrations of 0.8 $g \cdot g^{-1}$ and 0.7 $g \cdot g^{-1}$, and the binomial 95°C for 5 minutes the one with the highest propensity to exhibit higher values of viscosity. This similarity among the results obtained for ∫/t with viscosities PV, RV and FV is deduced when analyzing the correlation coefficients shown in Table 7.

For WPC analysis of variance for PV revealed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum value of PV obtained in the study was 3892 mPa·s on the condition of 0.4 $g \cdot g^{-1}$ at 85°C for 30 minutes and the minimum was 26 mPa·s with 0,1 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The variance analysis for RV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for the WPC. The maximum value of RV obtained in the study was 2086 mPa·s on the condition of 0.4 $g \cdot g^{-1}$ at 95°C for 5 minutes and at the minimum was 2mPa·s with 0.1 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The analysis of variance for FV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.005$) for WPC. The maximum value obtained in the study was 10105 mPa.s under the condition of 0.4 $g \cdot g^{-1}$ at 85°C for 15 minutes and the minimum was 6mPa·s with 0.1 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The magnitude of the effects of concentration and thermal processing on PV, RV and FV were statistically significant ($P < 0.05$), being the concentration of 0.4 $g \cdot g^{-1}$ and binomial 95°C for 5 minutes and 85°C for 15 minutes the most likely to show higher values of viscosity. The analysis of variance for ∫/t showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). These results demonstrate the influence of these variables on viscosity obtained after retention. The maximum value of ∫ / t obtained in the study was 3062 on condition of 0.4 $g \cdot g^{-1}$ at 85°C for 15 minutes and the minimum was 11 with 0.1 $g \cdot g^{-1}$ subjected to 65°C for 30 minutes. The magnitude of the effects of concentration and thermal processing on PV, RV and FV can be verified by ∫/t, demonstrating to be statistically significant ($P < 0.05$), with a concentration of 0.4 $g \cdot g^{-1}$ and the binomial 95°C for 5 minutes and 85°C for 15 minutes the most likely to exhibit higher values of viscosity. This similarity among the results obtained for ∫/t with viscosities PV, RV and FV is deduced when analyzing the correlation coefficients shown in Table 7.

In this study, we can infer that increases in viscosity observed in different simulations of thermal processing had no direct contribution on denaturation of casein molecules, since they have almost no secondary or tertiary structures. As described by [12], caseins do not suffer denaturation by heating at temperatures below 100°C, which occurs only at higher temperatures. This way, one can assign the increased viscosity of the solutions of MPPs during processing to thermal denaturation of whey protein and their association with the casein micelles. A common behavior in many assessed simulations was PV > RV, ie, the mean peak viscosities were higher than the average of the viscosities of retention. We can attribute this loss of viscosity to two factors. The first is the physical stress to which the solutions are subjected during the whole time of analysis (15.7 $rad \cdot s^{-1}$), causing the protein structure to lose part of its ability to interact with water due to the mechanical energy applied and the flow of product within the tub for heating. The second is related to the aggregation of β-LG and also

aggregate formation of β-LG/α-LA that will occur at a rate associated with duration of exposure to high temperature. During the retention time, the whey proteins will unfold in a short period of time, thus presenting opportunity for more monomers of unfolded β-LG self-aggregate [13], therefore decreasing the interaction with water, thereby lowering the viscosity of the solution.

During the cooling step, another common effect to various tests was substantial increase in viscosity until the final temperature of 30°C. The increased viscosity of the protein solutions during cooling is an attributed behavior not only to the decrease in molecular mobility due to decreased energy system, but also to the whey protein combinations to the surfaces of casein micelles during thermal processing, modifying their characteristics, and forming larger structures more hydrophilic, being capable of influencing the functionality of some dairy protein products [1].

The 3^3 Box-Behnken factorial design was conducted in simulated conditions with the WPC in RVA and Table 8 presents a coded matrix and results. Analyzing the results in Table 8, it appears that the highest value obtained for FV (8578 mPa·s) was provided on the condition of 0.4 $g \cdot g^{-1}$ at 95°C for 15 minutes and the lowest FV (29 mPa·s) on the condition of 0.2 $g \cdot g^{-1}$ at 65°C for 15 minutes. These results demonstrate the influence of these three variables in the thermal denaturation of whey protein of WPC. One FV of 8293 mPa·s close to the maximum achieved in prior condition was on the condition of 0.4 $g \cdot g^{-1}$ at 85°C for 5 minutes, showing that it is possible to optimize the application of energy and keep a final equivalent result in aspect of the desired viscosity. Regression analysis by response surface for final viscosity was performed using full quadratic model. The estimators of population parameters of the model were calculated by the minimum squares method X and Y matrices used to determine the estimators are shown in Fig. 2.

Table 9 shows calculated values for the coefficients and p-values for the adjusted model. Using a significance level of 4%, it is considered that a factor affects the response if the coefficients differ from zero or the p-value is less than 0.04 [14].

The results presented in Table 9 show that the terms: Temperature, concentration, temperature versus temperature, time versus concentration and temperature versus concentration are significant for FV at a level of 96% of confidence. In this way, all the variables affect the results of final viscosity. The model adjustment was assessed by analysis of variance (ANOVA). The adjusted regression model does not have the lack of adjustment in the confidence level of 96%, since the p-value was of 0.046 value for the ratio between the mean square due to the lack of adjustment and the quadratic mean and due to the pure error with three and two degrees of freedom, respectively. Thus it was possible to obtain nine response surfaces for the final viscosity model as represented in Figs. 3, 4 and 5. It is possible to observe that the highest final viscosity values come in different regions, depending on the variable that is used as a constant. Analyzing the models with constant concentrations of WPC (Figs. 3, 4 and 5) at the lowest concentration (0.2 $g \cdot g^{-1}$), the highest values of FV are obtained in the high level region for the time and for medium and high temperature (+1, 0, +1) (Fig. 3). On the average level of concentration (0.3 $g \cdot g^{-1}$), it is possible to obtain higher values of FV (Fig. 4) in the three time levels and in the medium and high levels of temperature (-1, 0, +1; 0, +1). Considering the high level of concentration (0.4 $g \cdot g^{-1}$), one obtains the highest values of FV (Fig. 4) in the region of negative and positive levels respectively for time and temperature (-1, +1). Similar analyzes can be made for models with constant processing temperatures (Figs. 3, 4 and 5). In the three temperature levels (65°C, 85°C and 95°C), the highest values FV (Figs. 3, 4 and 5 respectively) are obtained in the regions of negative levels for the time and positive levels for the concentration (-1, +1). As it can be seen in the models for the constant concentrations, in these models the FVs also have the tendency to increase in absolute values as the temperature levels vary from the lowest (65°C) to the highest (95°C). In all three areas where time was kept constant (Figs. 3, 4 and 5), the highest values of FV are obtained in the regions of positive levels for the temperature and concentration (+1, +1). In these cases, there is an inverse tendency of the behavior of FV in relation to the models of constant temperature. A decrease in the absolute values of the final viscosities occurs according to the variation of the levels of time from the lowest (5 min) to the highest (30 minutes). This behavior can be attributed to the denaturation kinetics of whey protein. In the positive levels of temperature and concentration, the WPC solution with 0.4 $g \cdot g^{-1}$ of dry basis in water in a condition of 95°C has a great effect of

thermal denaturation of whey protein due to high temperature and high concentration of proteins.

The interactions of various reactive groups such as thiol, or the hydrophobic moieties exposed on the surface of protein molecules, leading to the formation of covalent or non-covalent intermolecular bonds, which result in protein and/or polymerization aggregation. Such molecular changes alter the functional characteristics of proteins such as hydration, solubility, solution viscosity, film formation, and gelation adsorption at the interface between aqueous and lipid phases [3].

The rapid and significant increase in the viscosity of solutions of the MPPs in RVA during heating, resulting often in tilting viscosity can be explained with respect to denaturation of whey proteins to be considered as cooperation changes: The rupture of some of the bonds within molecule causes the remaining bonds to become less stable and many are broken simultaneously. This explains why the denaturation occurs in a very narrow range of temperature and concentration [12].

[15] Showed how the kinetics of thermal denaturation of whey proteins has a significant effect at temperatures above 90°C, especially in β-LG and α-LA. The change in protein conformation increases the available surface area and exhibits peptides and amino acid side chains, before hidden that in turn can interact with water [16] thereby increasing the viscosity. However, due to absence of other proteins such as caseins, self-aggregation of β-LG and aggregation of β-LG/α-LA occur at a rate associated with the time of exposure to high temperature. Thus, it is observed that this type of interaction has a negative effect on the increase of viscosity. At higher temperatures and faster heating rates, all whey proteins of milk start to unfold in a short period of time, thereby presenting more opportunity for the unfolded β-LG monomers self-aggregate [13] and thus a decrease in interaction with water is expected.

With the aim of obtaining an exploratory analysis by looking for patterns with all MPPs, the results of the three types of heat processing were organized in three different matrices, with treatments (MPP type and concentration) lines and the average readings of viscosity of the entire viscographic profile in the columns. Therefore, we obtained the matrix 1(24 x 375), matrix 2 (24 x 490) and matrix 3 (24 x 646) for the high, medium and low heat treatments respectively, 'number of treatments x number of mean viscosities read'. Assays were identified in the order that they are presented in Table 10.

Table 7. Correlation coefficients for area per unit of time (∫/t)

Variables for correlation (∫/t)	SMP r	MPC1 r	MPC2 r	WP r	DWP r	WPC r
Peak viscosity – PV	0.980 (*)	0.564 (*)	0.984 (*)	0.962 (*)	0.975 (*)	0.967 (*)
Retention viscosity– RV	0.977 (*)	0.778 (*)	0.980 (*)	0.977 (*)	0.978 (*)	0.959 (*)
Final viscosity – FV	0.979 (*)	0.588 (*)	0.991 (*)	0.961 (*)	0.990 (*)	0.938 (*)

Where: (*) statistically significant (P<0.001); r = pearson correlation coefficient

Table 8. Coded matrix for 3^3 box-behnken design and results of final viscosity-FV (mPa·s)

Experiment	Time	Temperature	Concentration	Final viscosity (mPa.s)
1	-1	-1	0	150
2	1	-1	0	669
3	-1	1	0	3119
4	1	1	0	4380
5	-1	0	-1	1827
6	1	0	-1	2198
7	-1	0	1	8293
8	1	0	1	2752
9	0	-1	-1	29
10	0	1	-1	2139
11	0	-1	1	932
12	0	1	1	8578
13	0	0	0	3180
14	0	0	0	3188
15	0	0	0	2595

Time (minutes): (-1) 5; (0) 15; (+1) 30; temperature (°C): (-1) 65; (0) 85; (+1) 95; concentration ($g \cdot g^{-1}$): (-1) 0.2; (0) 0.3; (+1) 0.4

Table 9. Estimation of the final viscosity model–FV

Term	Final viscosity - FV		
	Coefficient	Standard error (±)	*p*-value
Constant	$2.99 \cdot 10^{3}$	$1.96 \cdot 10^{2}$	$4.29 \cdot 10^{-3}$
Time	$-4.24 \cdot 10^{2}$	$1.20 \cdot 10^{2}$	$7.19 \cdot 10^{-2}$
Temperature	$2.05 \cdot 10^{3}$	$1.20 \cdot 10^{2}$	$3.41 \cdot 10^{-3}$
Concentration	$1.80 \cdot 10^{3}$	$1.20 \cdot 10^{2}$	$4.46 \cdot 10^{-3}$
Time x Time	$-3.01 \cdot 10^{1}$	$1.77 \cdot 10^{2}$	$8.81 \cdot 10^{-1}$
Temperature x Temperature	$-8.78 \cdot 10^{2}$	$1.77 \cdot 10^{2}$	$3.83 \cdot 10^{-2}$
Concentration x Concentration	$8.10 \cdot 10^{2}$	$1.77 \cdot 10^{2}$	$4.46 \cdot 10^{-2}$
Time x Temperature	$1.86 \cdot 10^{2}$	$1.70 \cdot 10^{2}$	$3.89 \cdot 10^{-1}$
Time x Concentration	$-1.48 \cdot 10^{3}$	$1.70 \cdot 10^{2}$	$1.30 \cdot 10^{-2}$
Temperature x Concentration	$1.38 \cdot 10^{3}$	$1.70 \cdot 10^{2}$	$1.48 \cdot 10^{-2}$

$$X = \begin{array}{c} M \quad X_1 \quad X_2 \quad X_3 \quad X_1^2 \quad X_2^2 \quad X_3^2 \quad X_{12} \quad X_{13} \quad X_{23} \\ \begin{bmatrix} +1 & -1 & -1 & 0 & +1 & +1 & 0 & +1 & 0 & 0 \\ +1 & +1 & -1 & 0 & +1 & +1 & 0 & -1 & 0 & 0 \\ +1 & -1 & +1 & 0 & +1 & +1 & 0 & -1 & 0 & 0 \\ +1 & +1 & +1 & 0 & +1 & +1 & 0 & +1 & 0 & 0 \\ +1 & -1 & 0 & -1 & +1 & 0 & +1 & 0 & +1 & 0 \\ +1 & +1 & 0 & -1 & +1 & 0 & +1 & 0 & -1 & 0 \\ +1 & -1 & 0 & +1 & +1 & 0 & +1 & 0 & -1 & 0 \\ +1 & +1 & 0 & +1 & +1 & 0 & +1 & 0 & +1 & 0 \\ +1 & 0 & -1 & -1 & 0 & +1 & +1 & 0 & 0 & +1 \\ +1 & 0 & +1 & -1 & 0 & +1 & +1 & 0 & 0 & -1 \\ +1 & 0 & -1 & +1 & 0 & +1 & +1 & 0 & 0 & -1 \\ +1 & 0 & +1 & +1 & 0 & +1 & +1 & 0 & 0 & +1 \\ +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \end{array} \qquad Y = \begin{array}{c} V_F \\ \begin{bmatrix} 150 \\ 669 \\ 3119 \\ 4380 \\ 1827 \\ 2198 \\ 8293 \\ 2752 \\ 29 \\ 2139 \\ 932 \\ 8578 \\ 3180 \\ 3188 \\ 2595 \end{bmatrix} \end{array}$$

Where:

X_1 is the time factor;

X_2 is the temperature factor;

X_3 is the concentration factor;

X_1^2 is quadratic parameter for time;

X_2^2 is quadratic parameter for temperature;

X_3^2 is quadratic parameter for concentration;

X_{12} is the interaction between time and temperature;

X_{13} is the interaction between time and concentration;

X_{23} is the interaction between temperature and concentration.

Fig. 2. Matrices X and Y used to determine the estimators

The use of Principal Component Analysis made it possible for the joint evaluation of 9000, 11760 and 15504 experimental data and for the high, medium and low heat treatment respectively. This collection of data makes the information provided by PCA significant under a relevant analytical point of view. Fig. 6 shows the PCA scores in the first two components to the low heat treatment (matrix 3), representing 98.44% of the total variance of the data. With the projection of two main components, it is possible to observe the formation of different groups among the MPPs samples. In the positive quadrant for PC1 and PC2, it is observed that tests 4, 23 and 24 are in the same region determined by the first main component, which explains the greater variety of samples (85.16%). Respectively, we have the rehearsals 0.8 $g \cdot g^{-1}$ of dry matter of the SMP, 0.4 $g \cdot g^{-1}$ and 0.45 $g \cdot g^{-1}$ of dry matter from MPC2. In the positive quadrant for PC1and negative for PC2, it is seen the samples 19 and 20 (0.3 $g \cdot g^{-1}$ and 0.4 $g \cdot g^{-1}$ of dry matter of WPC) in the same region for PC2. The positive quadrant for PC2 and negative for PC1 presents tests 21 and 22 (0.3 $g \cdot g^{-1}$ and 0.35 $g \cdot g^{-1}$ of dry basis from MPC2) and in the negative for PC1 and PC2 all other experiments in a cluster.

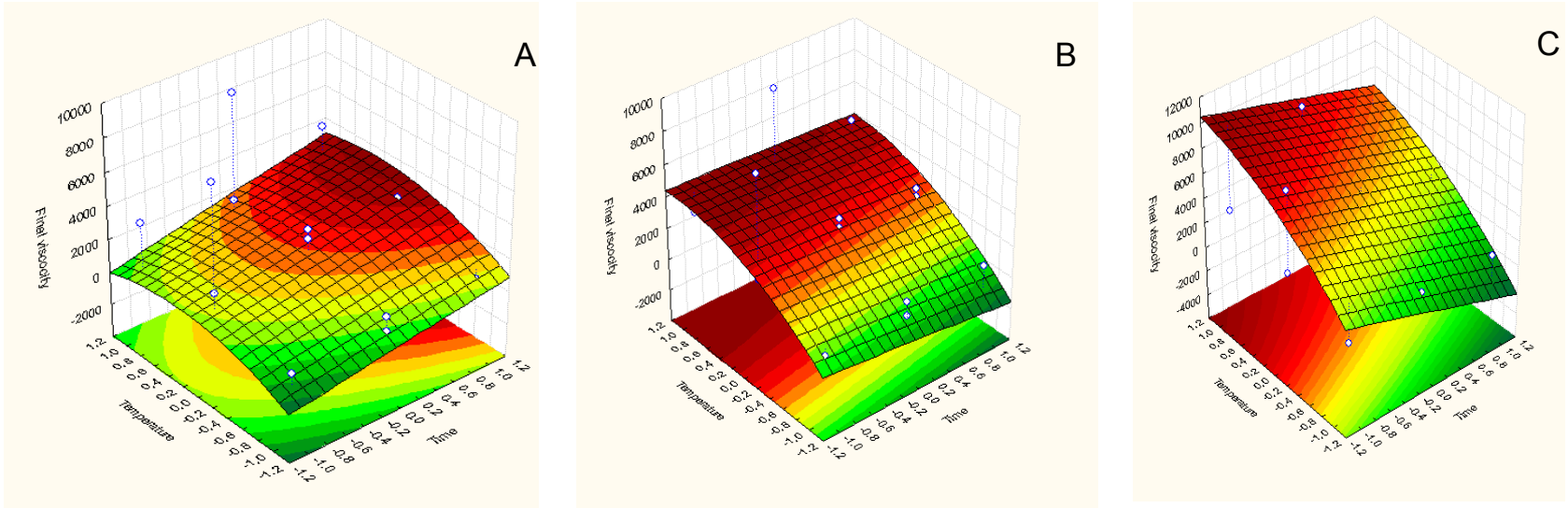


Fig. 3. Response surfaces obtained for final viscosity (FV) under the simulated conditions for WPC on RVA: (A) constant concentration on level -1 (0,2 g.g^{-1}), (B) constant concentration on level 0 (0,3 g.g^{-1}) and (C) constant concentration on level +1 (0,4 g.g^{-1})

*(A) z=2987.7-423.75*x-30.1*x^2+2054.5*y-878.1*y^2+185.5*x*y-1478.*(-1.)*x+1384.*(-1.)*y-985.3*
*(B) z=2987.7-423.75*x-30.1*x^2+2054.5*y-878.1*y^2+185.5*x*y-1478.*0.*x+1384.*0.*y+0.*
*(C) z=2987.7-423.75*x-30.1*x^2+2054.5*y-878.1*y^2+185.5*x*y-1478.*1.*x+1384.*1.*y+2605.2*

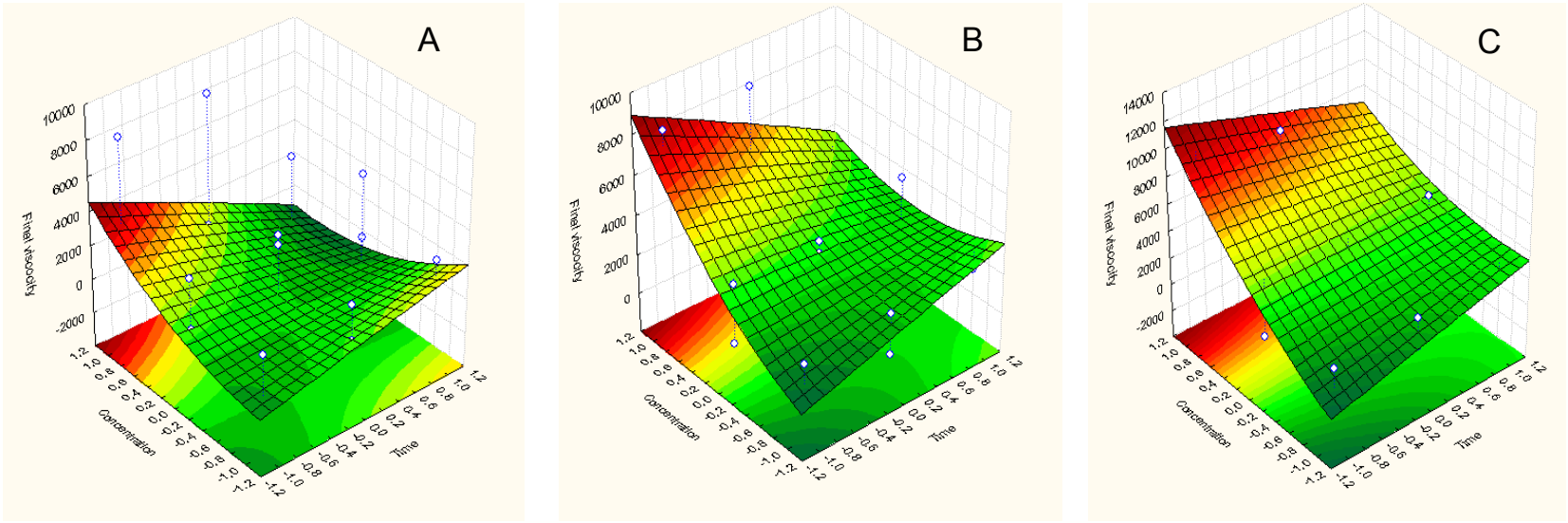


Fig. 4. Response surfaces obtained for final viscosity (FV) under the simulated conditions for WPC on RVA: (A) constant temperature on level -1 (65°C), (B) constant temperature on level 0 (85°C) and (C) constant temperature on level +1 (95°C)

*(A) z=2987.7-423.75*x-30.1*x^2+1795.25*y+809.9*y^2+185.5*(-1.)*x-1478.*x*y+1384.*(-1.)*y-2932.6*

*(B) z=2987.7-423.75*x-30.1*x^2+1795.25*y+809.9*y^2+185.5*0.*x-1478.*x*y+1384.*0.*y+0.*

*(B) z=2987.7-423.75*x-30.1*x^2+1795.25*y+809.9*y^2+185.5*1.*x-1478.*x*y+1384.*1.*y+1176.4*

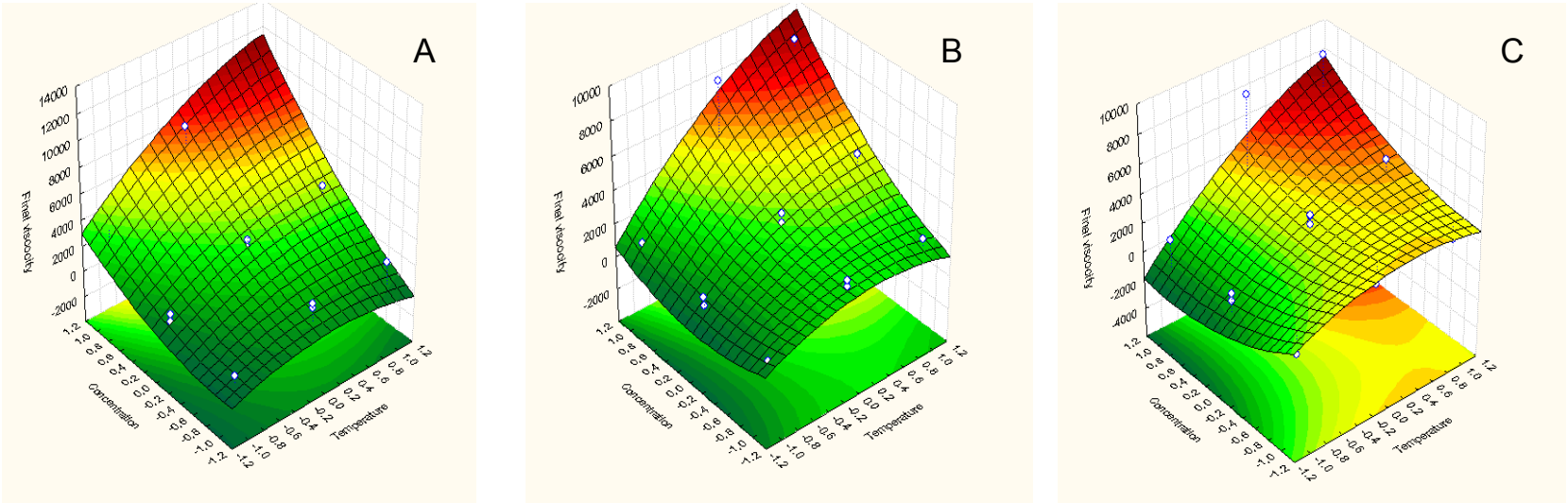


Fig. 5. Response surfaces obtained for final viscosity (FV)under the simulated conditions for WPC on RVA: (A) constant time under level -1 (5 minutes), (B) constant time under level 0 (15 minutes) and (C) constant time under level +1 (30 minutes)

(A) z=2987.7+2054.5*x-*878*.1*x^2+1795.25*y+809.9*y^2+185.5*(-1.)*x-1478.*(-1.)*y+1384.*x*y+393.7
(B) z=2987.7+2054.5*x-878.1*x^2+1795.25*y+809.9*y^2+185.5*0.*x-1478.*0.*y+1384.*x*y+0.
(C) z=2987.7+2054.5*x-878.1*x^2+1795.25*y+809.9*y^2+185.5*1.*x-1478.*1.*y+1384.*x*y-453.8

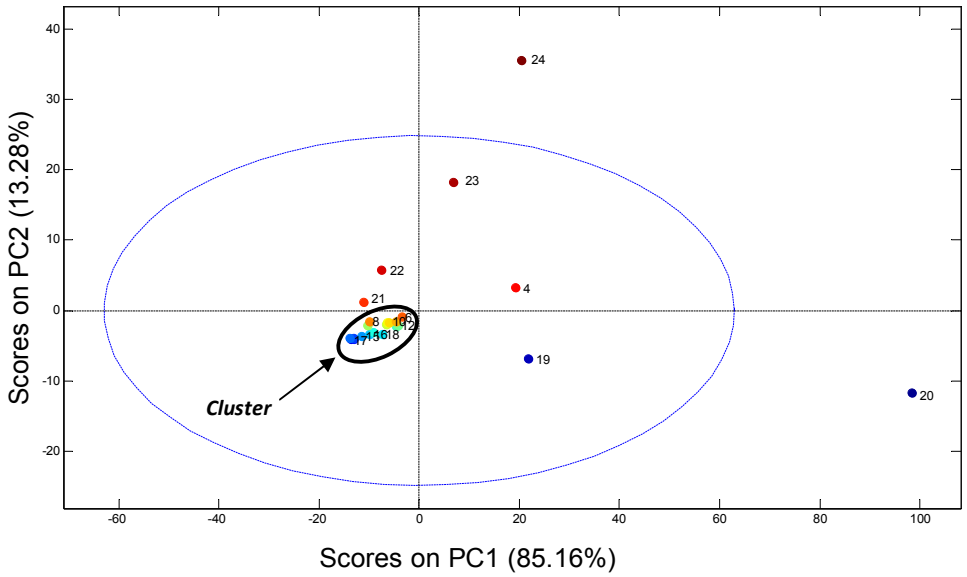


Fig. 6. Graphic of score for PC1 vs. PC2 under the low treatment conditions (65°C for 30 minutes). where: 4 is equal to SMP (0.29 g.g^{-1} of protein), 19 is equal to WPC (0.24 g.g^{-1} of protein), 20 is equal to WPC (0.33 g.g^{-1} of protein), 21 is equal to MPC (0.22 g.g^{-1} of protein), 22 is equal to SMP (0.26 g.g^{-1} of protein), 23 is equal to SMP (0.30 g.g^{-1} of protein), 24 is equal to SMP (0.34 g.g^{-1} of protein)

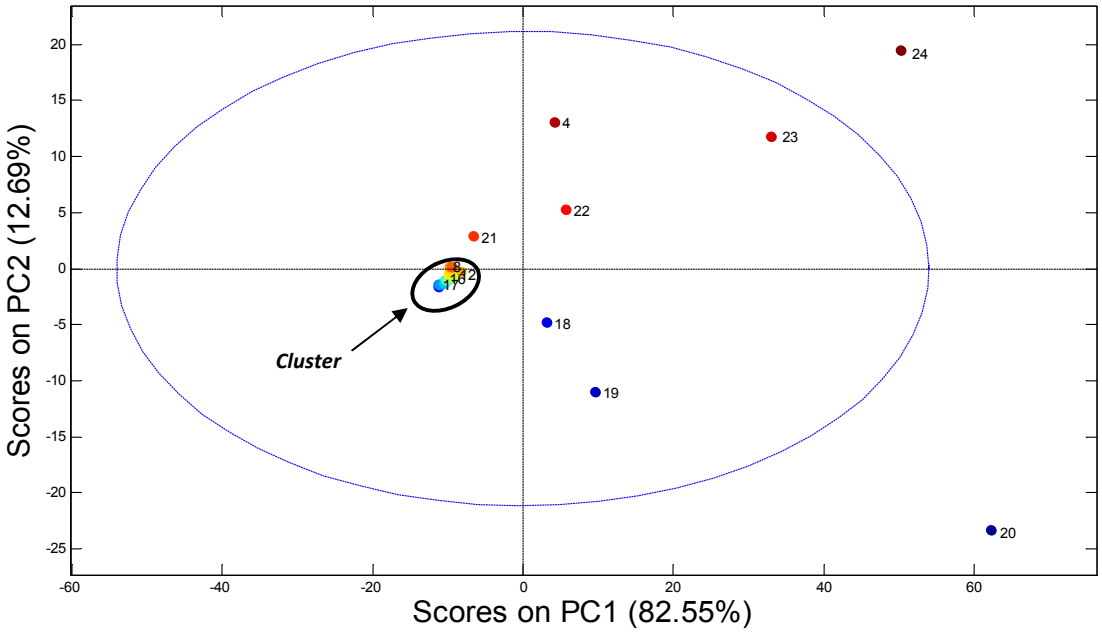


Fig. 7. Graphic of score for PC1 vs. PC2 under the medium treatment conditions (85°C for 15 minutes). where: 4 is equal to SMP (0.29 g.g^{-1} of protein), 19 is equal to WPC (0.24 g.g^{-1} of protein), 20 is equal to WPC (0.33 g.g^{-1} of protein), 21 is equal to MPC (0.22 g.g^{-1} of protein), 22 is equal to SMP (0.26 g.g^{-1} of protein), 23 is equal to SMP (0.30 g.g^{-1} of protein), 24 is equal to SMP (0.34 g.g^{-1} of protein)

Table 10. Identification of the experiments with MPPs on RVA

MPP type	Dry matter concentration ($g \cdot g^{-1}$)	Identification
Skimmed milk powder – SMP	0.5	1
	0.6	2
	0.7	3
	0.8	4
Milk protein concentrate – MPC_1	0.1	5
	012	6
	0.14	7
	0.16	8
Whey powder – WP	0.5	9
	0.6	10
	0.7	11
	0.8	12
Demineralized whey powder – DWP	0.5	13
	0.6	14
	0.7	15
	0.8	16
Whey protein concentrate - WPC	0.1	17
	0.2	18
	0.3	19
	0.4	20
Milk protein concentrate – MPC_2 (without prior hydration)	0.3	21
	0.35	22
	0.40	23
	0.45	24

It is possible to see in Fig. 6 that the PC1 identified the influence of low heat treatment on product solutions with the highest protein content and PC2 differentiated the influence of heat treatment on the origin of MPPs (milk or whey), except for those involved in cluster due to the low protein concentration. The protein content of separation in PC1 for MPPs originated form of milk (upper right quadrant of the graph) was 0.29 $g \cdot g^{-1}$ of protein for SMP and 0.30 $g \cdot g^{-1}$ protein to MPC2, close values considering the difference in concentration of dry matter of trial 4 (0.8 $g \cdot g^{-1}$ dry matter from SMP) and 23 (0.3 $g \cdot g^{-1}$ dry matter from MPC2). For the whey originated, the content of separation in PC1 was 0.24 $g \cdot g^{-1}$ protein from WPC, indicating a higher influence of the low heat treatment on the components of WPC over the SMP and MPC2. Tests 4, 19 and 24 are in the same region of PC1, indicating their similarity in viscographic characteristics, these being SMP, WPC and MPC2, respectively. Fig. 7 shows the PCA scores in the first two components to medium thermal treatment (matrix 2), representing 95.24% of the total variance of the data. With the projection of two main components, it is also possible to observe the formation of different groups among the MPPs samples.

For the medium heat treatment, in the positive quadrant for PC1 and PC2, it is observed that besides the trials 4, 23 and 24 which were already in the low heat treatment, the test 22 is also presented in the same region determined by the first main component for the SMP at 0.8 $g \cdot g^{-1}$ of dry matter. It is also observed, displacement of the experiments 23 and 24 for the right side of the quadrant, thus clarifying the influence of heat treatment on average MPC2 for the concentration of 0.4 $g \cdot g^{-1}$ and 0.45 $g \cdot g^{-1}$ of dry matter. In the positive quadrant for PC1 and negative for PC2, it is also seen the insertion of a further test (number 18, WPC 0.16 $g \cdot g^{-1}$of dry weight) besides the 19 and 20 (0.3 $g \cdot g^{-1}$ and 0.4 $g \cdot g^{-1}$ dry matter from WPC) in different regions for PC2. The positive quadrant for PC2 and the negative for PC1 shows only the test 21 (0.3 $g \cdot g^{-1}$ of dry matter MPC2) and in the negative for PC1 and PC2 all other tests in a cluster. It is possible to see in Fig. 7 that the PC1 continued identifying the influence of heat treatment on product solutions with the highest protein content and PC2 differentiating the influence of heat treatment on the origin of MPPs (milk or whey), except for those involved in cluster due to low protein concentration. The protein content of the separation in PC1 for the MPPs originated from milk (upper right quadrant of the graph) was 0.29

$g \cdot g^{-1}$ protein from SMP and 0.26 $g \cdot g^{-1}$ of protein for MPC2, indicating that the MPC2 presents greater influence of heat treatment compared to the average SMP for the concentration ranges studied. For those originated of whey, the content of separation in PC1 was 0.16 $g \cdot g^{-1}$ protein WPC, indicating a higher influence of the medium heat treatment on the components of the product compared to the low thermal processing. These three tests are in the same region of PC1, indicating similarity in the viscographic characteristics of them. The Fig. 8 shows the PCA scores in the first two components to the medium thermal treatment (matrix 1), representing 94.59% of total variation of data. With the projection of two main components, one can observe the formation of different groups among the MPPs samples.

The provision of test scores in the graph to the high heat treatment does not differ much compared to medium heat treatment. However, it is possible to notice similarities among the trials 4, 22 and 18 (respectively 0.8 $g \cdot g^{-1}$ of dry matter from SMP, 0.35 $g \cdot g^{-1}$ dry matter from MPC2, and 0.2 $g \cdot g^{-1}$ of dry matter from WPC), and also tests 23 and 19 (0.4 $g \cdot g^{-1}$ of dry matter from MPC2 and 0.3 $g \cdot g^{-1}$ of dry matter from WPC) in PC1.

It is possible to see in Fig. 8 that the PC1 continued identifying the influence of the heat treatment on the solutions of products with higher protein content, determining, inclusive, similarity of the evolution of the viscosity among the SMP, MPC WPC at concentrations of 0.29 $g.g^{-1}$, 0.26 $g \cdot g^{-1}$ and 0.16 $g \cdot g^{-1}$ of protein respectively, besides the MPC and WPC 0.30 $g \cdot g^{-1}$ and 0.24 $g \cdot g^{-1}$ protein also for PC1. The products, which were arranged in the cluster, did not have their influence characteristics of the heat treatment well defined due to the low protein concentration, indicating that this method of analysis explains better for the products of high protein in the solution, refer to the solutions of MPC1 are also attached to the cluster. For the three thermal processing, it was possible to use the PCA graphs to identify if the origin of the MPP was of milk or whey in solutions of high protein content. It was also possible to notice the similarities of the influences of heat treatment on the development of viscosity in solutions of the SMP, MPC2 and WPC and, making it possible to determine the protein concentration required to obtain the same evolution of viscosity among the solutions of these products.

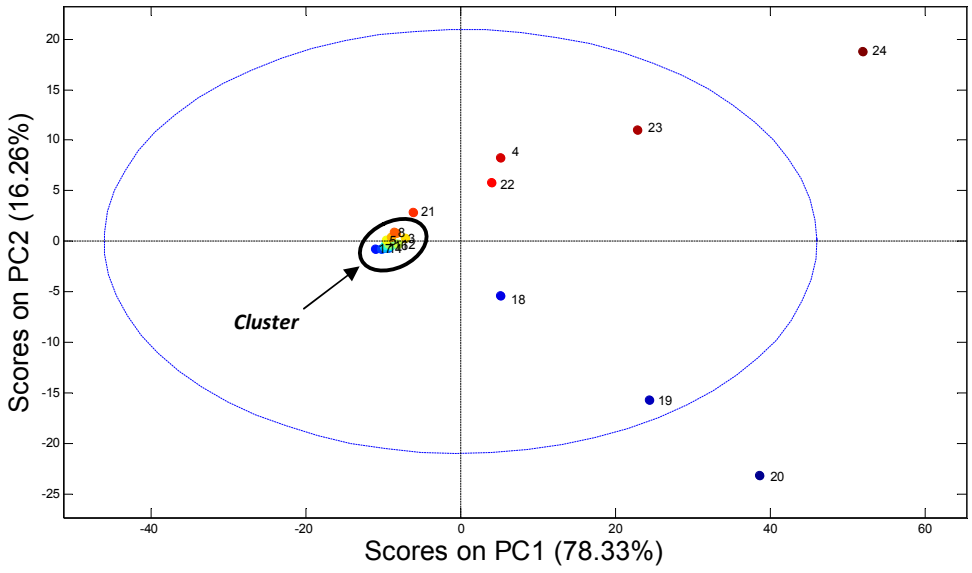


Fig. 8. Graphic of score for PC1 vs. PC2 under the high treatment conditions (95°C for 5 minutes). where: 4 is equal to SMP (0.29 $g.g^{-1}$ of protein), 19 is equal to WPC (0.24 $g.g^{-1}$ of protein), 20 is equal to WPC (0.33 $g.g^{-1}$ of protein), 21 is equal to MPC (0.22 $g.g^{-1}$ of protein), 22 is equal to SMP (0.26 $g.g^{-1}$ of protein), 23 is equal to SMP (0.30 $g.g^{-1}$ of protein), 24 is equal to SMP (0.34 $g.g^{-1}$ of protein)

4. CONCLUSION

Based on this work and the results obtained, it can be concluded that:

- The contents of dry matter and protein contributed to the increased viscosity of the solutions of MPPs, when subjected to thermal processing;
- The variables of time and temperature of heat treatment influenced the viscographic profiles, the viscosities peak, retention and final solutions of MPPs;
- The MPPs viscographic profiles were adequate to reveal the characteristics of functionality during and after the heat treatment;
- The principal components analysis of the viscographic profiles during thermal processing solutions have demonstrated that the MPP was originated from milk or whey;
- The RVA was convenient for the simulation of thermal processing of solutions for MPPs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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